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NEPHROLOGY FORUM

Lupus nephritis and the anti-phospholipid antibody syndrome in pregnancy

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CASE PRESENTATION

A 32-year-old woman was gravida 2, para 0, and had had one spontaneous abortion. When she was 12 weeks pregnant, she presented to the emergency department at the Brigham and Women's Hospital with new-onset nephrotic syndrome and systemic lupus erythematosus. Seven years earlier, she had had swelling in the small joints of her upper and lower extremities. At that time she was told that she had rheumatoid arthritis. Hydroxychloroquine sulfate (Plaquenil) relieved her symptoms. After five years of treatment, the patient discontinued taking the Plaquenil, as she was concerned about becoming pregnant. She did very well after the medication was stopped.

Approximately two years before this admission, she became pregnant. Other than a positive rapid plasma reagin, all antenatal tests were in the normal range. At approximately eight weeks gestation, the patient had a spontaneous abortion. She underwent a dilatation and curettage because of hemorrhage and was diagnosed with a septate uterus. The septum was hysteroscopically resected one year prior to the current admission.

The patient had been well until approximately eight weeks into her current pregnancy, when she developed facial, hand, and leg edema. The edema gradually worsened, with a 12 pound weight gain over the next four weeks. She also noted increasing shortness of breath, abdominal distension, and decreased appetite. One day prior to admission, the patient saw her primary physician, who noted that she was moderately hypertensive, with a blood pressure of 140/80 mm Hg as compared to a blood pressure of 110/70 mm Hg at six weeks of gestation. He also noted 3+ proteinuria on dipstick. A 24-hour urine collection contained 9 g of protein; the creatinine clearance was 102 mL/min. Serum creatinine had risen to 1.6 mg/dL from 1.0 mg/dL earlier in the pregnancy. ANA was positive at 1:320. Following this visit, the patient developed vaginal bleeding and presented the next day to the emergency department at the Brigham and Women's Hospital.

In the emergency room, the patient had no active vaginal bleeding. The medical history was noteworthy for a diagnosis of rheumatoid arthritis, hypothyroidism with a multinodular goiter, and mitral valve prolapse. She was taking levothyroxine sodium (Synthroid), 0.05 mg daily. She reported having one sister with an unspecified autoimmune disorder.

Physical examination revealed a patient comfortable at rest. Her blood pressure was 138/78 mm Hg and she was afebrile. Her weight was 182 pounds. Head and neck examination was remarkable for facial edema. No lymphadenopathy was apparent, and the joints and skin were normal. Cardiac examination was normal. Lung examination revealed decreased breath sounds one-half way up with dullness to percussion in the right hemithorax. The abdominal examination revealed distension consistent with ascites. The uterus was not palpable. The extremities showed 2+ pitting edema bilaterally. Her calves were not tender. A pelvic examination did not reveal bleeding.

The O_2 saturation was 98% while breathing room air. A chest radiograph revealed a right pleural effusion and right chest wall subcutaneous emphysema. An ultrasound study demonstrated an intrauterine pregnancy at 12.5 weeks, ascites, and a right pleural effusion. A urinalysis showed a specific gravity of 1.025; pH, 6.0; 3+ protein; and 3+ blood; the rest of the parameters were negative. Sediment examination showed 25 to 30 white blood cells/high-power field and too-numerous-to-count red blood cells/high-power field with 5 to 7 coarse granular/degenerating cellular casts. The blood urea nitrogen was 35 mg/dL and the serum creatinine was 1.6 mg/dL. Electrolytes were in the normal range. A complete blood count showed a hematocrit of 25.8%, white cell count of 1536/mm^3 (normal, 4000–10,000/mm^3), and platelet count of 128,000/mm^3 (normal, 150,000–450,000/mm^3). Liver function tests were normal. Her total protein was 3.3 g/dL (normal, 6.0 to 8.0 g/dL); albumin, 1.4 g/dL (normal, 3.7 to 5.4 g/dL); calcium, 6.8 mg/dL (normal, 8.8 to 10.5 mg/dL); phosphorus, 4.6 mg/dL (normal, 2.4 to 5.0 mg/dL); magnesium, 2.7 mg/dL (normal, 1.8 to 6.4 mg/dL); and uric acid, 6.5 mg/dL. Serum cholesterol was 259 mg/dL (normal, 140 to 240 mg/dL); the triglyceride level was

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379 mg/dL (normal, 35 to 135 mg/dL). Serologic tests showed a C3 of 84 mg/dL (normal, 88 to 201 mg/dL); C4, 21 mg/dL (normal, 12 to 36 mg/dL); CH50, 128 U/mL (normal, 150 to 250 U/mL); and a Clq of 27 mg/dL (normal, 8 to 12 mg/dL). The anti-double stranded (DS) DNA antibody was 78 IU (normal, 0 to 25 IU); anti-sm antibody, 4 EU/mL (normal, 0 to 20 EU/mL); anti-Ro 4 EU/mL (normal, 0 to 20 Eμ/mL); ANA, 1:640; and a negative ANCA. Her anti-cardiolipin antibody titer was <22 GPL units (normal, <15). The lupus anticoagulant screen was negative.

A provisional diagnosis of lupus nephritis with nephrotic syndrome was made. The patient was treated with prednisone, 60 mg/day, and furosemide, 40 mg/day. A renal biopsy was deferred because of the pregnancy. Over the next five days, the patient had further vaginal bleeding, and her hematocrit fell to a nadir of 20%. The serum creatinine peaked at 1.8 mg/dL, and a 24-hour urine collection revealed 12 g of protein. After much discussion, the patient elected to terminate the pregnancy on the seventh day following admission. Subsequently, the ascites and peripheral edema progressively improved. Prednisone was continued at the same dosage and she was discharged from the hospital.

A percutaneous renal biopsy was performed approximately 10 days following termination of the pregnancy. The biopsy consisted of two cores of renal tissue containing a total of 21 glomeruli. Five glomeruli showed periglomerular cellular or fibrocellular crescents. All glomeruli were remarkable for diffuse endocapillary cell proliferation. Few open loops were present. Numerous "wire-loop" lesions were present and frequent capillary loops revealed thickened walls with double contours. Occasional polymorphonuclear leukocytes were identified within the glomerular capillary tufts, and rare areas showed evidence of cellular damage with karyorrhectic debris. Most glomeruli showed a segmental increase in mesangial matrix and cells. The interstitium showed a few foci of mononuclear cell infiltration. There was focal mild interstitial fibrosis as well as focal mild tubular atrophy; however, most tubules were arranged in a normal back-to-back configuration with full epithelium. Mild thickening of arteries and arterioles was apparent. The activity index was 16, with a maximal score of 24. The chronicity index was 3, with a maximal score of 12. The sections were examined for reaction with antibodies against IgG, IgA, IgM, C3, albumin, fibrinogen, and kappa and lambda light chains. Twelve glomeruli were present on the examined sections. Diffuse granular reactivity was identified within glomerular tufts with antisera to IgG (3+/4+), IgA (2–3+/4+), IgM (1+/4+), and kappa/lambda light chains (trace to 1+/4+). Granular C3 (2+/4+) was identified within glomeruli in a diffuse segmental manner. The reactivity was found along glomerular capillary loops and most likely in mesangial areas. Small, segmental, confluent areas of fibrin/fibrinogen reactivity (3+/4+) were identified within some glomeruli. Linear albumin (trace) was identified along all basement membranes. Two glomeruli were examined by electron microscopy. Numerous subendothelial electron-dense deposits were identified. Rare subepithelial and more frequent mesangial deposits also were found. No specific structures were identified within the deposits. Visceral epithelial cells showed obliteration of foot processes. Diffuse mesangial cell proliferation also was evident.

Based on the renal biopsy, a diagnosis of WHO class-IV diffuse proliferative lupus nephritis was made. The patient was counseled about the potential effects of cyclophosphamide on future fertility, and she elected to undergo further treatment. One month following the onset of her nephritic illness, she was given pulse intravenous cyclophosphamide therapy (1 g intravenously each month) while continuing to take prednisone, 60 mg/day. Her therapeutic regimen comprised six monthly pulses of intravenous cyclophosphamide followed by four pulses of cyclophosphamide, one every three months. Her cyclophosphamide treatment has since been discontinued (cumulative dose, 10 g over 18 months). Her prednisone dose has been tapered gradually.

Over the past five years, she has experienced no further renal or extrarenal manifestations of lupus. Her renal function has remained stable. One year following her acute illness, the serum creatinine was 0.8 mg/dL. An iothalamate test of glomerular filtration rate showed 100 mL/min/1.73 m^2, and a 24-hour urine collection contained 423 mg protein. Four years following her acute illness, the serum creatinine remained at 0.8 mg/dL, with a creatinine clearance of 141 mL/min and a 24-hour urine protein excretion of 233 mg. At last follow-up, approximately five years following her acute illness, her serum creatinine was 0.9 mg/dL with a creatinine clearance of 120 mL/min and undetectable amounts of protein in a 24-hour urine collection.

DISCUSSION

Dr. Ajay K. Singh (*Clinical Director, Renal Division, Brigham and Women's Hospital; and Associate Professor of Medicine, Harvard Medical School, Boston, Massachusetts, USA*): Systemic lupus erythematosus (SLE) and the anti-phospholipid antibody syndrome (APS) are autoimmune syndromes that occur most commonly in women during their reproductive years. Forty years ago, approximately one of every 1660 pregnancies involved a mother with SLE. The frequency of pregnancy in lupus patients is now severalfold higher for a variety of reasons, including the emergence of immunosuppressive agents to better manage lupus, the marked improvement in obstetric care, and the realization among patients and physicians that pregnancy is not contraindicated in SLE. Today's case presentation highlights a number of questions that will be the focus of my discussion. First, what is the effect of pregnancy on the immune system and how is it of relevance to patients with SLE? Second, how does pregnancy affect SLE activity? In particular, what is the lupus flare rate during pregnancy, and does pregnancy exacerbate SLE activity? Further, does the clinical severity of lupus at onset of pregnancy predict the probability of further worsening of disease during that pregnancy? Third, what is the effect of SLE on pregnancy? In particular, how does SLE affect maternal and fetal outcome? Finally, what is the role of the anti-phospholipid antibody syndrome on pregnancy and fetal outcome, and how should APS be managed?

Immunologic adaptations in pregnancy

Among the many physiologic adaptations that occur during pregnancy, the adaptation of the immune system in the mother to a semi-allogeneic fetus is, perhaps, the least well understood. This immunologic balancing act allows the mother to avoid mounting a vigorous immune response to the presence of paternal major histocompatibility (MHC) antigens, and yet allows her to maintain

normal immune competence for defense against microorganisms. Although there are no significant differences in the absolute numbers of T- and B-lymphocytes and NK cell numbers, strong evidence demonstrates a switch from a Th1 to a Th2 response in pregnancy [1], as well as several potentially important functional adaptations. Comparisons of the mixed-lymphocyte reactions (MLR) of normal pregnant women and their non-pregnant, HLA-identical sisters indicate that, despite the presence of fetal (paternal) HLA antigens, maternal MLR are neither stimulated nor suppressed [2, 3]. Studies also report either normal or reduced NK cell cytolytic activity as well as suppression of lymphocyte proliferation. These alterations appear to be modulated by a 34 kD protein produced by lymphocytes named the progesterone-induced blocking factor (PIBF), which both influences lymphocyte and NK function and modulates the Th1 to Th2 switch [4, 5]. Because production of immunoglobulin and circulating levels of IgG, IgM, and IgA also are normal, B-cell function is likely intact. The mother does produce antibodies against some fetal (paternal) HLA antigens beginning at around eight weeks of gestation [6]; however, these autoantibodies are non-cytotoxic and regulated by an idiotypic network [7] that facilitates control of the immune response. Thus, pregnancy manifests selective functional changes in maternal immune responses that include a switch from Th1 to Th2 and a dampening of the response to fetal (paternal) HLA antigens.

The maternal immune response during pregnancy has been investigated with respect to both rheumatoid arthritis and SLE. Rheumatoid arthritis tends to remit rather than flare in pregnancy [8, 9]. This salutary effect of pregnancy on the disease activity appears to be modulated by a maternal-fetal disparity in HLA-DR and DQ antigens [8] as well as a switch from Th1 to Th2 T-cell responses. Unlike investigations into rheumatoid arthritis, studies in murine lupus models suggest that a switch to Th2 responses favors further activation of the immune response in SLE. Thus, the predominance of Th2 responses in pregnancy might explain why lupus tends to flare during pregnancy.

Immunologic adaptations during pregnancy are influenced by major endocrine changes, which might be relevant to pregnancy-associated lupus activity. Progesterone, estrogen, and androgen levels increase markedly during pregnancy. These changes are at first orchestrated by the corpus luteum, which makes progesterone during the initial six to eight weeks of gestation. Subsequently, progesterone is synthesized and secreted by the placenta. In addition to progesterone, the feto-placental unit is a major source of production of estrogen via a pathway involving the fetal adrenal gland and the compounds dehydroepiandrosterone and dehydroepiandrosterone sulfate. Maternal concentrations of progesterone and estrogen increase to four to eight times the level observed in the non-pregnant state. The effects of these compounds on the immune system are complex. As I alluded to earlier, progesterone suppresses lymphocyte proliferation by modulating the Th1/Th2 cytokine balance [reviewed in 9] through the release by lymphocytes of PIBF [8]. Progesterone-induced blocking factor then mediates the immunomodulatory effects of progesterone through the induction of Th2-type cytokines by activated lymphocytes. In addition, progesterone-induced lymphocytes produce soluble factors that favor the synthesis of prostaglandin E, which in turn might suppress IL-2 production from both NK cells and T-lymphocytes. Estrogens, on the other hand, stimulate an increase in maternal hepatic protein synthesis, which raises the concentration of a variety of serum proteins. Although estrogen is stimulatory for T-cell responses and autoantibody production, the fact that neither T-cell function nor immunoglobulin production increases dramatically during pregnancy suggests counterbalancing influences [10]. These counterbalancing immune suppressive factors include progesterone, α-fetoprotein, a pregnancy-specific β1-glycoprotein, pregnancy-associated α_2-macroglobulin, and human chorionic gonadotropin [10]. Whether progesterone-induced Th2 activity modulates lupus during pregnancy is still open to debate. Studies of pregnant lupus patients matched with non-pregnant controls suggest that pregnancy does not exacerbate clinical lupus activity [11, 12], but this remains a controversial issue. On the other hand, there appears to be agreement that lupus that is active at the onset of pregnancy is activated further during pregnancy, as evidenced by either clinical activation of disease, worsening of serologies, or both. One explanation for this difference could be that lymphocytes, after being activated during a pre-pregnancy exacerbation of disease, become more responsive to Th2 cytokines during pregnancy. According to this hypothesis, pregnancy does not induce flaring of lupus, but already active lupus can worsen during pregnancy because of the Th2 bias in immune responses.

The mechanisms underlying maternal tolerance to the semi-allogeneic fetus at the feto-placental interface also are beginning to be understood. Rejection of the trophoblast is prevented through the regulated expression of major HLA antigens by placental trophoblast cells [13]. Transcription and translation of the highly polymorphic class-I HLA-A, -B, and -C genes, whose products play a critical role in graft rejection, are blocked in trophoblast cells. In place of HLA-A, -B, and -C, trophoblast cells express HLA-G, a nonclassic, nonpolymorphic major histocompatibility complex class-I molecule, which is expressed in a restricted fashion on trophoblasts. Expression of HLA-G also appears to inhibit NK cell activity, as well as T-cell function. The role of HLA-G has been discussed elsewhere in detail [14]. While trophoblast cells do not express class-II HLA-D antigens, they do express

Fas ligand, thereby conferring immune privilege. Furthermore, trophoblasts express the complement regulatory proteins CD46, CD55, and CD59 [15]. A number of cytokine responses also appear to be important in implantation [16]. Interferon-γ, which usually increases HLA expression, appears to have no effect on trophoblast cells in situ. On the other hand, increased expression of cytokines, including IL-1, TNF-α, IFN-γ, GM-CSF, and CSF-1, influence blastocyst attachment, trophoblast outgrowth, implantation, proliferation of trophoblast cell lines, and placental function. Uterine decidual and placental cells also bias the Th response towards Th2. Finally, the placental barrier restricts the traffic of cytotoxic cells to the fetus, and cytotoxic antibodies are removed by the placenta before they reach the fetal circulation.

The balancing of immunologic and endocrine factors ensures the success of pregnancy. In essence, there are two battlegrounds: systemic, where tolerance of the maternal immune system to fetal (paternal) antigens in the circulation occurs, and local, at the feto-placental interface. Some evidence indicates that the level of disparity between maternal and fetal antigens (of the major HLA antigens) in pregnancy influences immune responsiveness, at least in patients with rheumatoid arthritis. Whether this finding is applicable to SLE remains to be seen. Studies from murine models suggest that the time of greatest likelihood for activation of lupus is the "childbearing" period (rather than pregnancy itself) because the immune stimulatory effect of estrogen is unopposed at this age. In the NZB/W murine model of SLE, reduction in the estrogen stimulus or an increase in the androgenic influence is associated with amelioration in disease activity [17]. In addition, sporadic evidence links exogenous estrogen therapy with the precipitation of a lupus flare. This issue is reviewed elsewhere in greater detail [18]. However, as I said earlier, to the extent that autoimmune activity proximate to the onset of pregnancy (<6 months) is associated with a greater flare rate during pregnancy, it is likely that the level of autoimmune activity at the onset of pregnancy influences subsequent disease activity during gestation.

Effect of pregnancy on lupus activity

The potential impact of pregnancy on lupus activity has been debated for decades. Bridge and Foley concluded in a 1954 article that "there is no constant effect of pregnancy on this disease" [19]. A large number of studies have since examined this issue, but no consensus has emerged. The lupus flare rate, examined in numerous studies [11, 12, 20–24], ranges from approximately 20% to 60% (Fig. 1). Retrospective studies invariably have noted an increased flare rate in pregnant women with lupus. However, these studies have significant limitations, including variable definitions of a lupus flare. For example, in one study reporting a flare rate of 22%, lupus flare was not defined at all [20]. In contrast, in several other studies (in which the flare rate ranged from 37.5% to 54.0%), clinical criteria defined a lupus flare [21–23]. Retrospective studies also have been dogged by small numbers of patients (as few as eight patients in one study [22]), non-random selection of subjects, use of patients with more severe underlying disease, and either the lack of a control group or the use of patients as their own controls. In studies in which carefully matched non-pregnant patients were used as controls, no excess flare rate was noted with pregnancy [11, 12], whereas in studies in which patients themselves functioned as controls, an excess flare rate was documented [21, 24]. The main problem with self-controls is that signs of active disease can diminish over time in patients with SLE. Furthermore, the use of historical controls rather than concurrent controls or the use of "published flare rates in other populations" has obvious limitations. Studies that report a relatively low rate of lupus flare in the control population also are not necessarily representative of the observed rate in other centers, and comparisons among studies might not be valid. In some studies, the level of disease activity at the onset of pregnancy has been poorly documented. Single-center studies are also open to biases. For example, studies from a high-risk obstetric center can comprise patients who have a history of more severe lupus—and therefore more likely to flare—compared with those from less-specialized centers where the patients might have less-severe disease.

Prospective controlled studies unfortunately have also generated conflicting results. Lockshin et al reported that the lupus flare rate is approximately 20% and found no difference in the flare rate when pregnant lupus patients are compared with matched non-pregnant lupus controls [11]. In contrast, Petri and coworkers reported a flare rate of approximately 60% and suggested that pregnancy exacerbates lupus activity [24, 25]. Several factors might explain this large discrepancy between prospective studies from experienced centers. The most important factor, as is the case with retrospective analyses, appears to be a lack of consensus on the definition of a lupus flare, even in carefully designed, prospective studies. Worsening of disease activity along a continuum, worsening of one aspect of lupus (for example, nephritis), or intensification of prednisone dosing all have been cited as "flares." But increased activity of a clinical abnormality or laboratory dysfunction is not always attributable to lupus. For example, worsening of proteinuria or hypertension could reflect changes attributable to pregnancy itself rather than to lupus. Facial and palmar erythema, thrombocytopenia, and effusions also are commonly a feature of a normal pregnancy, but their presence could be misinterpreted as a flare.

Another factor that might explain a higher flare rate is the quality of matching of pregnant patients with their

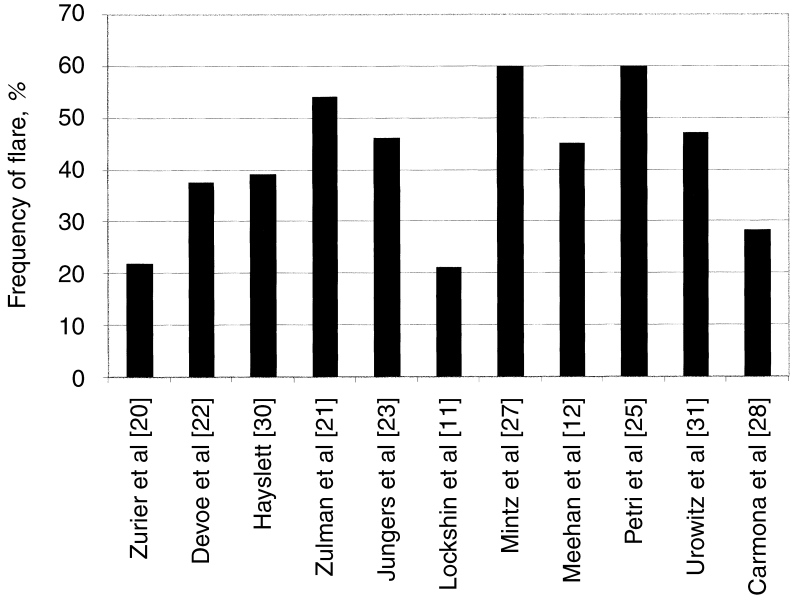

Fig. 1. Frequency of a lupus flare (in percentage) for 11 retrospective and prospective studies spanning three decades. The rate ranges from 20% to 60%. The citation number is given in parentheses.

controls. In the Petri studies, differences in baseline characteristics such as age (older patients in the control group), race (more African Americans in the control group), and the prednisone dose (higher in the non-pregnant control group) also could explain the higher flare rates among the pregnant lupus patients as compared with controls [25]. Follow-up of control patients was also different, that is, less frequent in the control group compared with the pregnant lupus patients. Finally, inclusion of patients who developed SLE during the index pregnancy could be considered controversial, because, strictly defined, de novo onset of lupus does not represent a flare of lupus per se; thus the association of lupus and pregnancy could have occurred by chance.

Studies reporting the absence of an excess lupus flare rate in pregnancy, on the other hand, also have been criticized. In Lockshin's study, lupus patients appeared to have mild disease—as evidenced by the relatively small proportion of patients taking prednisone (~40%) and the incidence of flares among the non-pregnant lupus controls—and the population might not have been representative of the general lupus population [11]. The absence of a significant difference in the frequency of flares in the study and control populations could simply reflect a sample size inadequate to detect this anticipated effect. Nevertheless, despite the limitations of the various studies, patients seek guidance about the likelihood of a flare during pregnancy. From my review, the best available data suggest a 20% to 60% likelihood of a lupus flare, depending on how one defines the severity of lupus in the patient and the criteria for diagnosing activity of the underlying disease at conception. Whether pregnancy is in fact associated with a higher risk of a lupus flare remains controversial. Lockshin's prospective data with well-matched controls suggest not.

Several factors present prior to the onset of pregnancy have been proposed as predictive of whether SLE will flare during pregnancy. These include presence of disease activity at the time of conception, duration of remission prior to the onset of pregnancy, and pattern of SLE activity in multiple pregnancies. In a study of 79 pregnancies in 36 patients, Estes and Larson suggested 35 years ago that the level of SLE activity at the time of conception is critically important [26]. In patients with active SLE, exacerbations of lupus during pregnancy occurred more than twice as often as remissions, and these exacerbations were most frequent in the post-partum period. More recent studies have not reported a higher rate of lupus flares in the post-partum period, however (Fig. 2) [21, 25, 27–29]. Estes and Larson also suggested that nephritis appeared to worsen progressively during pregnancy. Several reports have confirmed this observation [21, 30–32]. Using survey data, Hayslett and Lynn found that if SLE had been in clinical remission for at least six months prior to conception, 65% of the patients had a sustained remission, and only 32% had clinical evidence of a relapse. In contrast, active lupus at the time of conception was associated with increased or persistent lupus activity in 52% of patients [30]. A more recent study from Bobrie et al produced similar findings [32]. In the Bobrie study, lupus activity at conception also correlated with more severe flares and included progression to end-stage renal failure in some patients. Zulman et al examined the factors that predict SLE activity during pregnancy. They reported a clinical flare in 10 of 16 patients who had active renal disease

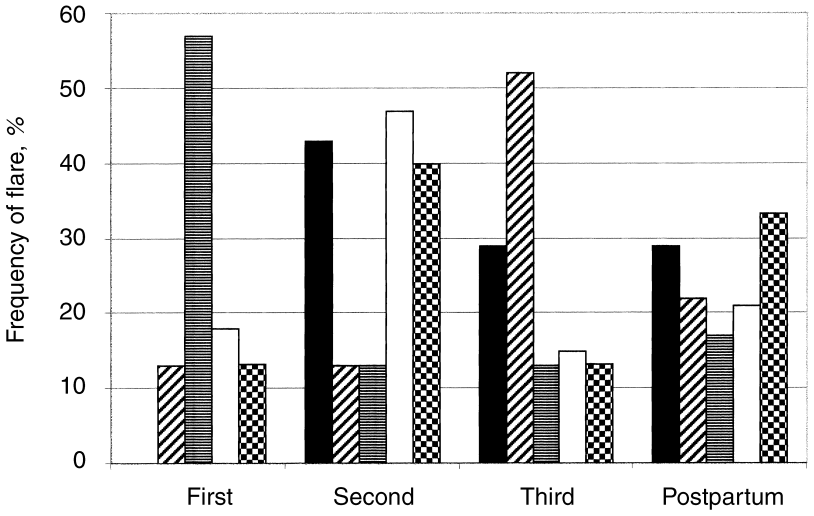

Fig. 2. Frequency of a lupus flare (in percentage) in the different phases of pregnancy and the postpartum period. There appears to be no consistent relationship between the phase of pregnancy and lupus flare activity. Symbols are: (■) Fraga et al [29]; (▤) Mintz et al [27]; (□) Petri et al [25]; (▨) Zulman et al [21]; (▩) Carmona et al [28].

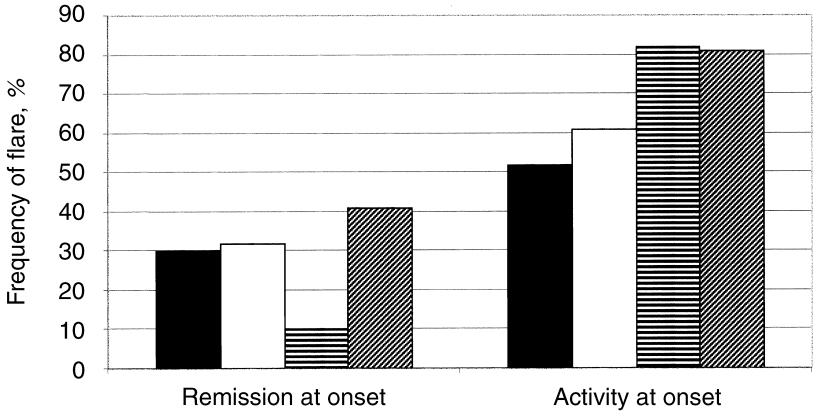

Fig. 3. Effect of disease activity on the subsequent rate of lupus flare. Remission at the onset of pregnancy appears to be associated with a lower flare rate than if SLE is active at conception. Symbols are: (■) Hayslett et al [30]; (□) Bobrie et al [32]; (▤) Tozman et al [39]; (▨) Urowitz et al [31].

but in only one of 6 without active renal disease prior to pregnancy. None of the patients without renal disease prior to pregnancy developed renal disease during pregnancy [21]. Although far from proven definitively, a reasonable body of evidence seems to support the contention that conception during active lupus, particularly in patients with active lupus nephritis, should be avoided (Fig. 3). A prudent rule of thumb based on the prevailing data is that SLE patients should wait at least six months after remission of lupus activity before becoming pregnant.

Effect of SLE on pregnancy and fetal survival

Systemic lupus erythematosus can influence pregnancy in several ways: induction of a pre-eclampsia-like syndrome, worsening of renal disease, and an increased risk of fetal morbidity and mortality. A lupus flare comprising thrombocytopenia, hypertension, azotemia, and sodium avidity is difficult to distinguish from pre-eclampsia [21, 33]. This is particularly true when pregnant lupus patients have circulating anti-phospholipid antibodies, because these patients commonly manifest thrombocytopenia. Complement levels sometimes are useful diagnostically but generally lack specificity. Normal pregnancy is marked by an increase in complement levels, particularly C3, C4, and CH50 [34]. However, low or normal complement levels can be difficult to interpret because some uncomplicated lupus pregnancies also are accompanied by low complement levels. To address problems with the specificity of hypocomplementemia in diagnosing a lupus flare, investigators have measured both complement and complement split product such as Ba, C3a, C4d, SC5b-9 and utilized ratios of these measurements (for example, a high CH50:Ba ratio). However, because assays measuring complement split products are not widely available in clinical practice, their value in managing a lupus pregnancy is limited.

Numerous reports have documented a high rate of adverse outcomes in pregnant patients with lupus [12, 23, 27, 30, 35–37]. These poor outcomes include a high rate of fetal loss, a high frequency of late miscarriages

and premature deliveries, and intrauterine growth retardation. Studies on fetal outcome are difficult to compare because they span a time frame that has witnessed major improvements in obstetric care, the relatively recent recognition of the role of anti-phospholipid antibody syndrome and the neonatal lupus syndrome in modulating fetal wastage, and the introduction of several therapeutic modalities to improve outcomes. Furthermore, studies have utilized different criteria in defining outcomes. For example, it is unclear whether fetal loss included late fetal deaths in utero, stillbirths, or deaths soon after birth. If one excludes studies prior to 1980, the rate of live births in pregnant lupus patients is 74% (fetal loss rate of 26%). This rate is consistent with data from a prospective study from France, which reported a live birth rate of 76% [35]. In contrast, in another prospective study, this one originating in China, a significantly lower live birth rate (58%) suggested a geographic variation perhaps related to differences in available technology [36]. The rate of spontaneous abortions is 18%, higher in patients with SLE than the rate in the general population but generally lower than figures pre-dating 1980. The main risk factors linked to fetal loss include preterm delivery, the presence of the neonatal lupus syndrome, active disease prior to or early in pregnancy, and the presence of abnormal renal function, proteinuria, or antiphospholipid antibodies in the patient. However, the literature lacks any comprehensive analysis that weighs the importance of these risk factors in determining fetal outcome.

Preterm delivery is a major influence on fetal morbidity in normal patients and in patients with lupus. The preterm delivery rate in lupus pregnancies varies extraordinarily widely among different studies, ranging from 13% to 74%. One potentially important factor that might explain this apparent discrepancy is the varying definitions of preterm delivery. In a study by Zulman et al, the reported rate was 13% [21]; however, Johnson et al reported a rate of 50% [38] and Imbasciati and colleagues reported a rate of 74% [37]. Causes of preterm delivery in the Johnson study included premature rupture of membranes (39.0%), pre-eclampsia or pregnancy-induced hypertension (32.4%), and spontaneous premature labor (11.8%) [37]. The mechanisms underlying the high rate of premature rupture of membranes in lupus pregnancies remain poorly understood. Indeed, in a relatively recent study, premature rupture of membranes did not seem to correlate with prednisone use, disease activity, or lupus serologic parameters [37].

How does pregnancy affect the severity of lupus nephritis? Retrospective and prospective studies have reported that patients with pre-existing renal disease have worse outcomes in terms of preterm delivery, incidence of pre-eclampsia, and worsening of proteinuria. The incidence of clinical renal syndromes and the frequency of various histologic lesions are open to non-random selection bias. Notwithstanding these legitimate concerns, Tozman et al reported that 11 of 18 patients had evidence of renal disease either clinically or on renal biopsy; 2 of the 11 had nephrotic syndrome [39]. The most common lesion (in 4 of 11 patients) was WHO class-II lupus nephritis. Unfortunately, the effect of pregnancy on either the clinical or laboratory parameters of renal disease was not documented. Zulman et al retrospectively analyzed their data and reported that of 24 pregnant patients, only 2 developed nephrotic syndrome during pregnancy. Notably, lupus flares occurred most commonly in patients who had renal involvement [21]. In a study by Rubbert and coworkers of 21 pregnancies in 19 lupus patients, 4 patients had renal involvement [40]. These patients had a worsening of proteinuria, from 1.3 to 3.7 g/24 hr to a range of 6 to 10 g/24 hr. All the patients had complicated obstetric outcomes, ranging from pre-eclampsia (2 of 4 patients), spontaneous abortion (one patient), and preterm delivery (one patient).

Neonatal lupus syndrome

The neonatal lupus syndrome is the most dramatic immunologic example of the maternal immune system transferring an autoimmune disease to the fetus. Only a small fraction of mothers with lupus or other rheumatic diseases deliver infants that develop neonatal lupus erythematosus (NLE) [41]. However, mothers who have circulating anti-SSB/La and SSA/Ro antibodies confer a high risk of NLE on the fetus [41]. Initially described by McCuistion and Schock in 1954 [42], NLE is the result of transplacental transfer of pathogenic anti-La/SSB and Ro/SSA autoantibodies from the mother to the fetus. The syndrome comprises transient cutaneous lupus erythematosus that presents as a generalized photosensitive rash, hematologic abnormalities including thrombocytopenia and anemia, giant cell hepatitis with severe cholestasis, and isolated complete heart block [43]. Rarely, nephrotic syndrome or a myasthenia-like presentation has been reported [44, 45]. Complete heart block is usually diagnosed in the second trimester, when fetal bradycardia is detected. Other cardiac manifestations include myocarditis, congestive heart failure, and a pericardial effusion; cardiac pathology is discussed in greater detail elsewhere [46]. Neonatal lupus erythematosus can occur as a part of, or precede the development of, the maternal autoimmune disease, most frequently SLE. In a study by Buyon and colleagues of children with congenital heart block, 34% of the mothers had SLE, 16% had Sjögren's syndrome, 22% had undifferentiated autoimmune disease, and 28% had no overt autoimmune disease [47].

The association between NLE and anti-SSA/Ro and/or anti-SSB/La in affected mothers and infants was first described in 1983 [48]. Since then, several groups have examined the pathogenetic, structural, and genetic origins

of the putative pathogenic autoantibodies. Several lines of evidence support a pathogenetic role for anti-SSA/Ro and anti-SSB/La antibodies in NLE. These include observations demonstrating the reactivity of maternal immunoglobulin to fetal heart tissue, the correlation between maternal antibody titer and clinical illness in the child, and experiments using rabbit hearts perfused with anti-SSA/Ro and anti-SSB/La sera [reviewed in 49]. There also appears to be a close correlation between the presence of the anti-SSA/Ro antibodies and the presence of congenital heart block [43], but the precise mechanisms by which autoantibodies interfere with conduction in the fetal myocyte or Purkinje cell remain obscure.

Autoantibodies to SSA/Ro recognized a ribonucleoprotein complex composed of small single-stranded RNAs and one or more peptides [reviewed in 49]. Two molecular forms of this complex are prominently involved, a 52 kD peptide and a 60 D form. The Ro complex has a diverse tissue and cell distribution, including expression in erythrocytes and platelets. Its function remains unknown, although it might participate in RNA transcription processes. The immune response to the SSA/Ro antigen is heterogeneous. Anti-SSA/Ro-positive sera might contain antibodies recognizing either a 60- or a 52 kD polypeptide component of the SSA/Ro particle. Antibodies to the 52 kD SSA/Ro protein have at least two antigenic determinants, an "immunodominant" determinant, and a second region recognized by a more "restricted" subset of anti-52 kD SSA/Ro Abs [50]. The 60 kD protein has a molecular partner identified recently as a novel 75 kD protein (pp75), which localizes predominantly in the cytoplasm [51]. The heterogeneity of the anti-SSA/Ro response and its relationship with underlying rheumatic disease have been examined. The major responses are anti-52 kD antibodies in primary Sjögren's syndrome, both anti-52 and anti-60 kD antibodies in SLE, and anti-60 kD antibodies in rheumatoid arthritis and other connective tissue diseases [52]. There is no specific immunoglobulin subclass distribution of anti-48 kD SSB/La and anti-52 and -60 kD SSA/Ro antibodies in the maternal and neonatal circulation in affected mothers and infants with NLE [53].

The management of NLE involves several strategies, including aggressive preventive protocols, screening using two-dimensional and M-mode echocardiography, possible prenatal treatment with steroids, and early delivery. Maternal immunosuppression with steroids and plasmapheresis also were used with success in the 1970s and 1980s. Buyon and her group recommend that all mothers with anti-SSB/La or anti-SSA/Ro, particularly with the 52 kD form, undergo serial fetal echocardiography by an experienced pediatric cardiologist at 16, 18, 22, and 24 weeks of gestation [54]. They further suggest consideration of prenatal prophylactic steroids in high-risk patients (for example, mothers with the 52 kD component of anti-SSA/Ro). If the fetus has an abnormal echocardiogram, dexamethasone (because it is not metabolized by the placenta and is available in an active form to the fetus) and plasmapheresis have been suggested [54].

Anti-phospholipid antibody syndrome during pregnancy

Pregnancy-associated complications of the antiphospholipid antibody syndrome affect both the mother and the fetus. These include fetal death (which can occur early or late), intrauterine growth retardation, premature delivery, and dysmaturity. Consensus exists that the presence of antiphospholipid antibodies (APAs) in pregnant women is associated with a high risk of repeated abortions before the 20th week; the frequency ranges from 53% in a study by Branch et al [55] to 77% in a study by Prentice et al [56]. There is also a risk of unexpected intrauterine deaths in the second and third trimester; the frequency ranges from 22% in the Prentice study [56] to 46% in the Branch study [55]. In addition, the mother can suffer from venous and/or arterial thrombosis; thrombocytopenia; pregnancy-induced hypertension; chorea; multi-system organ failure, including renal, hepatic, and cardiac failure; and post-natal depression.

Although initially the APS was considered wholly a part of the SLE syndrome, it has become increasingly evident that it can occur as a primary syndrome. The primary antiphospholipid antibody syndrome (PAPS), described nearly two decades ago by Graham Hughes, is termed Hughes' syndrome in his honor [57]. Primary and secondary forms of APS have similar clinical profiles, with the exception of lower C4, autoimmune hemolytic anemia, endocardial valve disease, and neutropenia in secondary APS [58]. Both forms are associated with thrombosis and an increased risk of fetal wastage [58], although the frequency of fetal loss appears to be lower in patients with primary APS than with SLE-associated secondary APS.

Antiphospholipid antibodies comprise a family of immunoglobulins whose specificity has been defined by in-vitro assays. Initially the antigen was thought to be anionic phospholipids. However, data over the past several years indicate that these antibodies have variable specificities. Antigenic moieties include β2-glycoprotein I, human prothrombin, placental anticoagulant protein (PAP), thrombomodulin, tissue factor, and phospholipase A_2. Broadly, APAs can be regarded as a family of antibodies that, on the basis of in vitro assays, can be categorized into three groups, lupus anticoagulant (LA), anticardiolipin antibodies, and reagin antibodies. Since the original description of LA in 1952 by Conley and Hartmann, the presence of LA in the serum has been correlated with venous and arterial thromboembolism. Lupus anticoagulant interferes with one or more of the in-vitro phospho-

lipid-dependent coagulation assays, such as the APTT, prothrombin time, or dilute viper venom time (dRVVT). Anticardiolipin antibodies currently are measured in either a solid-phase ELISA or a radioimmunoassay and are directed predominantly against the β2-glyoprotein I moiety, which is a physiologic anticoagulant. The β2-GPI appears to inhibit the contact phase of coagulation and impedes the prothrombinase complex (factor Xa, factor Va, calcium ions, phospholipid, and prothrombin). Reagin antibodies react with the VDRL reagent. The VDRL reagent denotes a mixture of antigens, including cardiolipin, cholesterol, and lecithin. In contrast to the presence of other APAs, studies indicate that a false-positive VDRL test in pregnancy does not increase the risk of fetal loss. Studies characterizing the antiphospholipid antibody response report much heterogeneity in antibody specificities. Approximately one-third of patients with SLE have antibodies detected as lupus anticoagulant, and 40% to 60% of patients have anticardiolipin antibodies. In light of the spectrum of specificities and the growing evidence that combinations of antibodies of various isotypes and specificities correlate with specific clinical presentations, it is likely that future clinical workups of patients with APS will require assays that measure these different antibodies.

The most prevalent anti-phospholipid antibodies are anti-β2-GPI antibodies. "Pathogenic" anti-phospholipid antibodies, that is, those associated with disease, initially were perceived as being exclusively IgG in isotype [59]. However, recent work suggests that IgA and IgM isotypes also are associated with disease. Thus, anti-β2-GPI antibodies of IgG, IgA, and IgM isotypes were present in 84.8%, 59.3%, and 51.5% of patients with APS, and the frequency and level of each isotype were significantly higher in patients with APS [60]. A strong relationship also has been demonstrated between increased IgA anti-β2-GPI antibody levels and a history of venous thrombosis, thrombocytopenia, cardiac valve disease, livedo reticularis, and epilepsy. Likewise, IgG anti-β2-GPI antibodies, usually in association with the LA, are associated with the main features of APS. In contrast, antibodies of IgM isotype are related only to thrombocytopenia and cardiac valve disease. African Americans appear to have a higher frequency of IgA β2-GPI antibodies, so perhaps a genetic and/or racial predisposition for these antibodies is present [61]. Guglielmone and Fernandez examined the relationship between APS isotype and excess risk from pregnancy and found that the concurrent presence of IgG, IgM, and IgA, compared to the presence of a single dominant isotype among autoantibodies, seems to increase the frequency of recurrent spontaneous abortion [62].

The factors that influence the activity of APS during pregnancy, as well as the pathogenesis of fetal wastage in the antiphospholipid antibody syndrome, are only beginning to be understood. Evidence that Th1 cytokines suppress manifestations of APS in animal models raises the possibility that a Th1-to-Th2 switch during pregnancy makes mothers more vulnerable to APS [63]. Fetal loss appears to be the consequence of thrombosis of the uteroplacental vasculature as well as of placental infarction [reviewed in 64]. Antiphospholipid antibodies might induce a coagulant state by binding to antigens such as phosphatidylserine, thrombomodulin, and heparan proteoglycan on endothelial cells and trophoblast cell surfaces [65]. Indeed, in vivo studies in a murine model support a direct thrombogenic role for APAs [66]. Beta2-GPI appears to function as a cofactor that facilitates this crucial interaction. The resulting endothelial cell activation is associated with cell surface expression of E-selectin, vascular cell adhesion molecule (VCAM-1), and intracellular adhesion molecule (ICAM-1), and leads to monocyte adhesion, the first step in thrombosis. Although the precise mechanisms mediating endothelial cell-platelet interaction have not been fully elucidated, platelet binding to the endothelium appears to be the next phase in thrombosis. Potential targets for APA on platelets include platelet activating factor, phosphatidylserine, and platelet glycoprotein IIIa. Evidence thus suggests that APAs cause platelet activation and aggregation. Other abnormalities in the clotting system might conspire to further induce activation of the coagulation cascade. These include protein C and/or protein S deficiency [66, 67]. The target organ for the thrombotic events appears to be the spiral arteries of the placental bed.

A role for annexin V in APA-associated pregnancy loss has been suggested. Rand and Wu postulate that annexin V, which is expressed by placental and vascular endothelium, plays a thromboregulatory role at the vascular-blood interface by shielding anionic phospholipids from forming a complex with coagulation proteins in the circulation [68]. Thrombosis in the antiphospholipid syndrome is due to disruption of the annexin shield by antiphospholipid (and cofactor) antibodies, which results in the increased exposure of thrombogenic phospholipids. Several lines of evidence support this hypothesis. In a murine model, APAs rather than anti-β2-GPI antibodies per se appeared to be thrombogenic [69]. Annexin V displaced coagulation factors from phospholipid surfaces [70]. The level of annexin V was markedly reduced on placental villi in mothers with APS [71]. Furthermore, Rand et al reported in-vitro data showing that trophoblasts and endothelial cells exposed to antiphospholipid IgG had reduced levels of annexin V as compared to controls [72]. Furthermore, trophoblasts and endothelial cells exposed to APA IgG had significantly faster coagulation times [72]. Although this mechanism is a tantalizingly attractive explanation for thrombosis and fetal wastage in APS, it might not represent all the factors at play. For example, as I mentioned earlier, low plasma

concentrations of protein S [66] and mutations in factor V (factor V Leiden) in patients with APS have been reported [67]. Evidence in animal models—for example, the pinch-injury model using a femoral vein—suggests that endothelial damage is a prerequisite for thrombosis [73]. Furthermore, upregulation of adhesion molecules on endothelial cells and on platelets in APS models also has been reported.

The management of the antiphospholipid antibody syndrome during lupus has been transformed since the emergence of evidence that low-dose aspirin and heparin markedly reduce the fetal wastage rate. Interfering with the coagulation cascade rather than suppressing the immune response parallels the efficacy of anticoagulation in treating APS patients who are not pregnant [74]. Indeed, in a prospective randomized controlled trial, Rai reported an increase in the rate of live births to mothers given a low-dose aspirin and heparin combination from 42% to 71% as compared with births in women given aspirin alone [75]. These observations correlate with similar observations by other investigators [76, 77]. On the other hand, prednisone treatment in APA has not been effective in treating APS during pregnancy [78]. Although immunoadsorbent plasmapheresis or intravenous immunoglobulin has been used during pregnancy, the data are either sporadic or uncontrolled, and I do not recommend use of these approaches.

Conclusions

I have discussed various aspects of the lupus pregnancy. Despite more than 40 years of research in this area, and following the publication of several hundred papers, a lack of consensus exists on whether pregnancy exacerbates SLE. Still, we have made many advances in our understanding of the immunologic factors at play in a normal pregnancy and how these adaptations might affect autoimmune diseases such as lupus. The importance of factors that might influence a lupus flare at conception, including the presence of active disease, in particular lupus nephritis, have become well appreciated. The clinical and pathogenetic aspects of both the neonatal lupus syndrome and the anti-phospholipid syndrome also are coming into clearer view. Vigorous efforts are now focused on elucidating the precise mechanisms that underlie both the neonatal lupus syndrome and the antiphospholipid antibody syndrome.

QUESTIONS AND ANSWERS

Dr. Nicolaos E. Madias (*Executive Academic Dean, Tufts University School of Medicine, Boston, Massachusetts*): Is there any information on animal models of the antiphospholipid antibody syndrome?

Dr. Singh: Yes, a number of studies have explored the immunopathogenesis of the antiphospholipid antibodies through the use of animal models—mostly in mice but also in rabbits. As I alluded to earlier, the pathogenicity of APAs is due to their direct thrombogenic role. Evidence supports beta-2-GPI-mediated activation of endothelial cells followed by a series of downstream events that result in thrombosis. In vivo murine models support this direct role. For example, Garcia and coworkers have immunized PL/J mice with beta-2 microglobulin and reported the development of the antiphospholipid antibody syndrome, including adverse pregnancy outcomes, in the beta-2 microglobulin-injected mice [79]. Similarly, Shoenfeld's group, in numerous studies, have precipitated APA syndrome by injecting beta-2 microglobulin into naïve mice [80]. It is interesting that this group developed monoclonal APL antibodies using the combined method of EBV transformation and somatic cell hybridization of lymphocytes from patients with APS [81]. One monoclonal antibody, termed EY2C9, bound weakly to cardiolipin and other phospholipids, but when injected into naïve BALB/c mice, EY2C9 produced sustained high titers of antiphospholipid antibodies associated with prolonged activated partial thromboplastin time. Also, pregnant mice immunized with EY2C9 had increased fetal resorption rate (the equivalent of fetal loss in the human). Shoenfeld et al hypothesized that EY2C9 dysregulated the idiotypic network and precipitated the characteristic signs of APS. Other studies using anti-CD4 antibodies as well as other approaches have suggested that T-cells are important in immunopathogenesis.

Dr. Madias: Primary antiphospholipid antibody syndrome can cause a thrombotic lesion involving glomeruli as well as renal arterioles and arteries. Could you please comment on differentiating this lesion in a pregnant patient from severe pre-eclampsia and the relatively rare TTP-HUS?

Dr. Singh: Remarkably, studies documenting the renal pathologic manifestations of APA syndrome have been sparse. My colleague, Helmut Rennke, who coincidentally also described the pathology of the case presented today, discussed the pathology of the anti-phospholipid antibody syndrome in a recent CPC in the *New England Journal of Medicine* [82]. In addition, Nochy and coworkers have reviewed the typical histologic lesions found in the kidney in patients with the APA syndrome [83]. They report a vascular nephropathy characterized by small vessel vaso-occlusive lesions with fibrous intimal hyperplasia of interlobular arteries, recanalizing thrombi in arteries, and focal cortical atrophy.

Dr. Madias: What is known about other antigens against which antiphospholipid antibodies are directed?

Dr. Singh: As I alluded to in my presentation, antiphospholipid antibodies comprise a heterogenous family of antibodies. The specificity of these antibodies has been characterized by in-vitro assays. Good evidence suggests

several candidate protein antigens, including beta-2-glycoprotein I (beta-2-GPI), prothrombin, and annexin V [84]. The nature of these interactions is still being investigated. Antiphospholipid antibodies appear to recognize complexes of phospholipid and phospholipid-binding proteins, in particular, phospholipid and prothrombin or annexin V. The in vitro effect is inhibition of one or more of the in vitro phospholipid-dependent tests of coagulation. In vivo, these interactions can activate adhesion molecules on endothelial cells and downstream of this placental thrombosis in pregnant women. Of course, these antibodies also can precipitate one or more of the protean manifestations of the antiphospholipid antibody syndrome.

Dr. John T. Harrington (*Dean, Tufts University School of Medicine*): You mentioned that the pathogenesis of this syndrome involved the adherence of platelets to activated endothelial cells. Can we block the "stickiness" of the platelets in patients with this syndrome?

Dr. Singh: So far, the use of anticoagulation (warfarin therapy) or aspirin and heparin in pregnant patients is considered established therapy. To my knowledge, no studies have used anti-ICAM antibodies or, for that matter, strategies directed at the blockade of the platelet surface membrane receptors to reduce platelet aggregation. As you know, the glycoprotein IIb/IIIa receptor is in the news because it plays a pivotal role in binding circulating fibrinogen or von Willebrand factor and thereby crosslinking platelets to increase platelet "stickiness." In the cardiac arena, the chimeric monoclonal antibody fragment abciximab, the peptide inhibitor eptifibatide, and the nonpeptide mimetics tirofiban and lamifiban have been utilized with some success. I am not aware of trials in patients with the antiphospholipid antibody syndrome.

Dr. Harrington: Tell us about annexin. Why is it there and what does it do?

Dr. Singh: Annexin V is a member of the annexin family [84], which comprises anionic phospholipid-binding proteins previously known by names including placental anticoagulant protein, vascular anticoagulant, endonexin, lipocortin, calphobindin, calcimedin, calelectrin, and anchorin. Annexin V is expressed in a variety of tissues, although its presence in the placenta and vascular endothelium is currently most intriguing. It is a potent anticoagulant protein because it can displace coagulation factors from phospholipid surfaces. Rand and colleagues have investigated its role in pregnancy loss in patients with the antiphospholipid antibody syndrome because it is markedly reduced on placental villi in patients with this syndrome [85–87].

Dr. Harrington: Is it on the subsurface?

Dr. Singh: Annexin V is expressed on the apical membranes of syncytiotrophoblast cells isolated from the placenta.

Dr. Harrington: Is it simply a shield or does it have any specific physiological role?

Dr. Singh: To the best of my knowledge, the precise physiologic role for annexins remains unclear.

Dr. Ronald D. Perrone (*Division of Nephrology, New England Medical Center, Boston, Massachusetts*): You talked about normal pregnancy as a state of diminished immune response. Does lupus flare after pregnancy? Were you able to determine that from your review of the literature?

Dr. Singh: There is evidence on both sides of the fence about whether lupus flares in the immediate postpartum period. As I said in my presentation, several older studies do suggest postpartum flaring, but I found no reports of postpartum flaring in prospective controlled studies.

Dr. Madias: Is there evidence that increasing the dose of prednisone for a short period prior to and after delivery decreases the frequency of postpartum flares of lupus nephritis?

Dr. Singh: Not to my knowledge. However, a short course of steroids prior to and after delivery is quite safe, and I would not hesitate to use it if I thought that the patient were at high risk, for example, in a patient who already has evidence of serologic activity of lupus, or in someone who has evidence of a low-level flare.

Dr. Andrew J. King (*Division of Nephrology, New England Medical Center*): I'm interested in your construction of the pathogenesis of the increased risk of spontaneous abortion in patients with anti-cardiolipin antibodies. You alluded to an endothelial cell insult followed by expression of adhesion molecules and subsequent adhesion by platelets and initiation of thrombosis. Is there any evidence that inflammatory cells bind to adhesion molecules in this circumstance?

Dr. Singh: The cellular events that underlie pregnancy loss in the antiphospholipid antibody syndrome have not been completely elucidated. However, I believe that there is a very interesting study that specifically addresses your question. Pierangeli and coworkers recently used a microcirculation model in mice to demonstrate that antiphospholipid antibodies directly activate endothelial cells in vivo [87]. Moreover, endothelial cell activation can be correlated with in vitro expression of adhesion molecules (in particular VCAM-1 and E-selectin) on the surface of human umbilical vein endothelial cell (HUVEC) monolayers exposed to antiphospholipid antibodies. Thus, there does appear to be compelling evidence that endothelial cell activation influences hypercoagulability in patients with the antiphospholipid antibody syndrome. Nevertheless, how antiphospholipid antibodies activate endothelial cells remains a murky issue. One possibility is that antiphospholipid antibodies cause endothelial cell injury, but there are other possibilities.

Dr. King: Is there a breakdown of the immunoprivileged status that clearly exists in the placenta?

Dr. Singh: I'm not aware of any data that show that.

Dr. King: What do you postulate is the proximate cause of endothelial cell injury?

Dr. Singh: As I alluded to earlier, some evidence supports a role for direct injury to the vessel wall and presumably to endothelial cells as a prerequisite—for example, the pinch-injury model using a femoral vein [73].

Dr. Annamaria Kausz (*Division of Nephrology, New England Medical Center*): In pregnant women with lupus and renal failure, what is the impact of loss of the fetus on recovery of renal function? That is, can the loss of potential immune stimulation be separated from the effect of the heavy immunosuppression that was instituted when the pregnancy was lost? Would fetal loss alone have resulted in renal recovery?

Dr. Singh: Unfortunately, little data are available to address your question. If one accepts the postulate that pregnancy does not exacerbate lupus, then terminating the pregnancy should not be pursued. On the other hand, many sporadic reports suggest the contrary as well.

Dr. Klemens B. Meyer (*Division of Nephrology, New England Medical Center*): You suggested that the wide range of repeated flare rates, about 20% to 60%, represents variation across different populations. Might not this variability in small studies just reflect sampling variation? Perhaps the message is that the rate in the lupus population is on the order of 40%, the midpoint of that range. It seems to me that the order of magnitude is really what's important. What our patients need to know is that the flare rate is somewhere in this intermediate range, and that it is not 0.1%, 1%, 99% or 99.9%. I suspect that relatively few people would change their decisions depending on whether the rate is 20% or 60%.

Dr. Singh: I agree.

Dr. King: Your talk and the literature provide convincing evidence that disease activity at the beginning of the pregnancy predicts a flare. Can you offer us a clinical definition of pre-existing disease?

Dr. Singh: A reasonable definition of pre-existing disease is the presence of either extrarenal or renal manifestations of SLE coupled with serologic evidence of activity. Of course the more difficult issue is the asymptomatic patient who has evidence of serologic activity. However, as I discussed in my presentation, complement levels can be difficult to interpret in pregnancy.

Dr. King: What about the ANA?

Dr. Singh: I believe the ANA to be useful. But although it is sensitive, it has limited specificity. I find measuring anti-DNA antibody levels and complements very helpful.

Dr. Madias: In patients with quiescent lupus nephritis, does it matter what the underlying pathology is regarding a renal flare during pregnancy?

Dr. Singh: Yes, I believe that whether the patient has class III versus class IV to be very important. In fact, the distinction between class IIIa and IIIb is of substantial relevance because the latter is often associated with subendothelial immune deposition and behaves more like a class IV lesion in activity and outcome.

Dr. Steven Ralston (*Division of Maternal/Fetal Medicine, New England Medical Center*): This patient didn't have a renal biopsy because she was pregnant. Could you comment on the use of renal biopsy in pregnant women?

Dr. Singh: Yes, the patient discussed earlier did not have a percutaneous renal biopsy because she was pregnant. Although we usually shy away from performing a renal biopsy in pregnant women, under the appropriate circumstances a renal biopsy can be performed safely in a pregnant patient. I have performed several biopsies in pregnant women with nephritis, and although one must perform these biopsies with a great deal of caution, in every instance the information turned out to be invaluable.

Dr. Ralston: What do you do about a lupus patient who has mild renal disease with a slightly elevated creatinine at the beginning of pregnancy, but who at 24 to 26 weeks suddenly develops worsening proteinuria and a slightly increased creatinine?

Dr. Singh: Obviously, the differential diagnosis includes pre-eclampsia, exacerbation of pre-existing renal disease, and the de novo presentation of acute glomerulonephritis. After the appropriate workup, a renal biopsy could be entertained. One usually can make a diagnosis on clinical and laboratory parameters, however.

Dr. Dana Miskulin (*Renal Fellow, Division of Nephrology, New England Medical Center*): Have there been any studies of ARS patients who developed DVT during pregnancy that would help guide long-term therapy? Specifically, should these patients be anticoagulated over the long term?

Dr. Singh: I would choose low-molecular-weight heparin with aspirin as the preferred treatment.

Dr. Mark Sarnak (*Division of Nephrology, New England Medical Center*): You described one study using subcutaneous heparin, 5000 units twice daily, to prevent spontaneous abortion. In that study, 13 of 45 pregnancies still had poor outcomes. Has anyone used higher doses of heparin to prevent thrombotic events?

Dr. Singh: There have been other studies. Low doses of heparin appear just as effective as higher doses [88]. To specifically answer your question, Kutteh has used heparin in doses that ranged from 10,000 to 15,000 units subcutaneously to maintain the PTT at 1.2 to 1.5 times normal [89]. Supplementation with calcium carbonate also was provided to achieve a total daily intake of 1.5 g/day for all patients. This was to counter the problems with bone loss. In this study, the patients appeared to tolerate the heparin well, and no serious bleeding problems, episodes of thrombocytopenia, or osteoporotic

fractures were reported. Remarkably, 11 of 25 women treated with aspirin (44%) delivered viable infants as compared with 20 of 25 women treated with heparin and aspirin (80%) ($P < 0.05$).

Dr. Madias: How do the effects of lupus nephritis on the pregnancy compare with those of other glomerulopathies?

Dr. Singh: Jones and Hayslett examined the effect of pregnancy on pregnant patients who had a history of chronic glomerulonephritis or tubulointerstitial nephritis and mild to moderate renal insufficiency [90]. They noted a decline in GFR in 20% of patients, an increase in preterm delivery and caesarean section, but a high fetal survival rate—over 90%. I am not aware of an analysis that has systematically compared the lupus pregnancy with other glomerulonephritides.

Dr. Rekha Abichandani (*Renal Fellow, Division of Nephrology, New England Medical Center*): My first question is, which are the most common forms of lupus nephritis associated with the antiphospholipid antibody? My second question is, what do you do with patients who have not lost previous pregnancies and have an isolated antiphospholipid antibody?

Dr. Singh: Let me first respond to your question of whether to treat pregnant women considered to be at low risk of the antiphospholipid antibody syndrome, that is, women who have none of the associated signs or symptoms of the syndrome. This group has been evaluated by Cowchock and Reece, who concluded that treatment of pregnant women with antiphospholipid antibodies who are otherwise at low risk cannot be justified at present [91]. Regarding the most frequent class of lupus in pregnancy, as with non-pregnant patients, the answer depends on the setting. If one looks at patients referred to nephrologists, the answer is class IV disease. On the other hand, I would guess that if one were to survey pregnant patients with a history of lupus who do not necessarily have evidence of renal disease or who have mild proteinuria, the answer likely would be different.

Dr. Samina Khan (*Renal Fellow, Division of Nephrology, New England Medical Center*): What's the basic pathology of the placenta in these patients with lupus and APS? In both cases you get infarction.

Dr. Singh: Placental pathology is characterized by insufficiency and infarction. More specifically, Levey and colleagues reported thrombosis, acute atherosis, a decreased amount of syncytiovascular membranes, and an increased number of syncytial knots and obliterative arteriopathy [92].

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